Isolation of angiotensin converting enzyme from pig lung

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Angiotensin-converting enzyme (ACE) is an ectoenzyme anchored on the membrane of endothelial cells of blood vessels. The enzyme converts angiotensin I to angiotensin II, the latter is a potent vasoconstrictor and plays an important role in regulating blood pressure. Clinical studies have shown that oral administration of ACE inhibitors can effectively reduce blood pressure in hypertensive patients (1). ACE inhibitors have been reported in a number of food products (2) and there has been an increasing interest in possible usage of food-derived ACE inhibitors to prevent or treat human hypertension. To study food-derived ACE inhibitors, an enzymatic assay system for ACE is required. In this report, we describe a simple procedure for isolation of ACE from pig lungs.

Fresh pig lungs were purchased from the local market. After removal of large windpipes, the lung tissue was cut into pieces and minced. The minced tissue was then defatted by suspension and homogenization first in acetone and then in ether. Defatted tissue was dried in fumehood and then ground to powder. The tissue powder was suspended in 100 mM sodium borate buffer (pH 8.3) and mixed for 3 hours at 4°C. Tissue particles were removed by centrifugation at 40,000 g for 40 min and the supernatant as ACE crude extract was collected for further purification. The crude extract was further purified by ion exchange chromatography through a Q Sepharose Fast Flow Column (Pharmacia). The bound protein was recovered by eluting the column with 0.2 M NaCl in 100 mM sodium borate buffer (pH 8.3). After desalting through a PD-10 column (Sigma), the elute was freeze-dried to powder. Protein content in both crude extract and freeze-dried powder was determined by Lowry method and the ACE activity was determined by a photometric assay in combination with HPLC analysis (3).

	Protein Content (mg/mL)	ACE activity (U/mL)	ACE Specific Activity (U/g)
Crude Extract	51	0.24	4.6
Ion-exchange Extract	2.1	0.11	51.4
Freeze-dried Powder	440	24.1	54.8

About 80 g of defatted tissue powder was produced from 1 kg fresh pig lung tissue. For each gram of defatted tissue powder, 20 mg freeze-dried ACE extract powder was produced. As shown in the table, after a single step of purification by ion-exchange chromatography, ACE specific activity per unit of protein increased over 10-fold. The final extract contained 44% protein with a specific ACE activity of 54.8 U/g. Enzyme activity was inhibited by ACE specific inhibitor, Captopril, in a dose response dependent manner. Kinetic studies showed a Km value of 1.4 mM which is in agreement with the that reported in the literature. This report demonstrates a cheap source of ACE for further study.

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3. Meng QC, Balcells E, Dell'italia L, Durand J, Oparil S. Sensitive method for quantitation of angiotensin-converting enzyme activity in tissue. Biochem Pharmacol 1995; 50:1445-1450.